(1) scientific abstract: There are thirteen million adults in the United States- alone with symptomatic coronary artery disease. Antianginal drugs, the initial management of patients with stable angina, act by reducing myocardial oxygen demand or by increasing blood flow to the ischemic myocardium. Revascularization procedures are often necessary and consist of angioplasty (with or without stents) or coronary artery bypass graft (CABG) surgery. In 1996, 400,000 angioplasty procedures and nearly 500,000 coronary bypass graft procedures were performed in the U.S. Despite the increasing use of stents, restenosis remains a significant problem following angioplasty. Morbidity and high cost are significant disadvantages of CABG surgery. At the present time, transmyocardial laser revascularization procedure requires surgery.

The healthy human heart lacks native collateral vessels, but these (collateral vessels) may form in response to myocardial ischemia. These collaterals are in most cases, insufficient to meet increased blood flow required during stress. The process of coronary collateral vessel formation in response to ischemia is not well understood. Angiogenic proteins have been shown to be expressed in ischemic regions of the heart possibly originating from myocytes. The initial event in new vessel formation probably involves mitosis of capillary endothelial cells. There is, up to now, no approved therapy that will stimulate new blood vessel formation.

Using an ameroid ischemia model, Giordano et al performed gene transfer by an intracoronary delivery of a recombinant adenovirus expressing human fibroblast growth factor-5 in pigs. The results of this study indicate an improvement in regional LV function and blood flow to the ischemic area. Two weeks after intracoronary injection, there was also evidence of angiogenesis. This improvement was sustained at 12 weeks. A similar benefit in LV function and perfusion to the ischemic area was also seen with FGF4 gene transfer. Successful transgene transfer, expression and biological activity of the transgene product were demonstrated.

Two weeks after intracoronary injection of the recombinant adenovirus, Giordano et al were unable to detect viral DNA in liver, retina or skeletal muscle using polymerase chain reactions (PCR), despite the presence of transgene DNA in the myocardium. Pulmonary artery blood drawn during intracoronary injection of the recombinant virus contained less than 1.5% of the virus injected into the coronary artery. There was no evidence of myocardial inflammation, necrosis or fibrosis. The results of the above study demonstrate the effectiveness and safety of adenovirus-mediated angiogenic gene therapy in pigs.

The pig model of myocardial ischemia closely mimics the clinical situation in man. Both pigs and humans are devoid of native collateral vessels in the healthy heart. However, in response to myocardial ischemia, collateral blood vessels develop, but in those patients that remain symptomatic the collateral vessels fail to me-et the need for enhanced blood flow during stress. This clinical situation is mimicked by the pig model of myocardial ischemia. Collaterals develop, but are inadequate to meet myocardial demands during pacing induced stress.

The proposed clinical development will test whether gene transfer with human adenovirus-5 FGF-4 gene (Ad5FGF-4) in patients with exertional angina will augment collateral blood flow and relieve myocardial ischemia.

The gene product is an El A/B deleted human adenovirus serotype 5 with hFGF-4 insert driven by the CMV promoter. The gene product will be diluted with normal saline prior to administration.

The gene product will be administered by (once only) intracoronary injections in the left (left anterior descending and circumflex) and right coronary arteries. Sixty percent (60%) of the dose will be injected into the left coronary system and 40% of the dose in the right coronary system

Patients with stable angina will be evaluated in the clinical program. Clinical studies will be initiated to provide evidence that the gene product (by enhancing collateral formation) increases myocardial perfusion to ischemic areas, reduces stress-related myocardial ischemia and relieves angina. The studies will evaluate whether the above clinical benefit can be brought about without significant risks from adenoviral infections, neovascular growth or potential to exhilarate existing malignancies. Once developed collateral vessels should remain patent. Therefore, the clinical benefit from gene therapy should persist long-term.

Adenovirus mediated angiogenic gene therapy with Ad5FGF-4 is indicated for the long-term management of patients with stable angina

The Phase l/2 study of Ad5FGF-4 (Protocol 97166). which is ongoing, has two components:
(1)   The Main Study" (Protocol 97166 with amendments A and B) involves the evaluation of five amending dose groups (Ad5FGF-4 3.2×10^8 - 3.2×10^{10} viral particles) in stable angina patients who were symptomatic despite antianginal drug therapy. Characteristics of the patient population included CCS angina class 2-3, LVEF $\geq$ 40% and absence of significant heart failure (NYHA III and IV excluded).

(2)   The "Extension" (amendment C) allows the study of a few additional patients with patent bypass grafts. The inclusion criteria were also relaxed to allow entry of patients with LVEF > 30%, CCS class 2-4 (but patients had to be able to exercise using the modified Balke protocol for at least 3 minutes) and NYHA heart failure class (NYHA I, II and III).

Recruitment in this trial of Ad5FGF-4 in patients with stable angina has been completed but follow-up is ongoing. Eighty-nine (89) patients have been enrolled. Sixty-seven (67) patients were enrolled in the main study (Protocol 97166 and Amendments A and B) and all 67 have completed follow-up for at least 12 weeks.

In the main study, 67 patients received Ad5FGF-4 or placebo at doses of 3.2×10^8 - 3.2×10^{10} viral particles (VP). The ratio of placebo to active was 1: 3. Dose increase was by ½ log increments and the lower dose had to be safe before the next higher dose could be evaluated. In each dose group, two patients were enrolled at intervals and only if safety concerns were not encountered the remaining patients were enrolled.

All 67 patients have completed at least 12 weeks of allocated follow-up. In addition a further evaluation occurred at 6 and 12 months to record any significant clinical events and the angina status between 12 weeks and 6 months and 6 and 12 months. This evaluation is ongoing in some patients and the 6 and 12 month data will be formally reported separately.

In this Phase 1/2 multicenter double-blind (investigator and patient blind but sponsor unblinded for close monitoring of safety and for decision to increase sample size) safety study of Ad5FGF-4, 67 patients (mean age 59 years; 57 males) with stable angina, CCS class 2-3 and NYHA class l--2 with LVEF > 40% were enrolled. Most patients had CCS class 2 angina and NYHA heart failure class l--2. The mean LVEF was 60%, the mean time to angina (treadmill exercise using a modified Balke protocol) was around 6 minutes, the mean time to ≥ 1 mm ST segment change was around 7 minutes and the mean exercise duration was about 9 minutes. Except for duration of stable angina and percentage of patients with prior MI the active and placebo groups were well matched.

Five doses of Ad5FGF-4 (in ascending sequence) starting with 3.2×10^8 viral particles and increasing by ½ log increments to 3.2×10^{10} VP were administered via the intracoronary route (60% in the left and 40% in the right coronary systems). One patient who required immediate revascularization was randomized but did not receive treatment, All 67 patients were followed for at least 12 weeks. Randomization was undertaken in the ratio of 1 placebo to 3 active.

The 67 patients were randomized as follows:

		Placebo	Active
Dose group 1	3.2×10^8 VP	3	9
Dose group 2	10^9 VP	3	9
Dose group 3	3.2×10^9 VP	3	9
Dose group 4	10^{10} VP	5	13
Dose group 5	3.2×10^{10} VP	2	11
Total		16	51

- Gene product administration (3.2×10^8 VP -- 3.2×10^{10} VP) was not associated with hypotension, allergic reactions or significant arrhythmias.
- The 24-48 hour stay in hospital following study product administration was uneventful except in dose group 5 (3.2 x 10E10 VP) where 3 of the 9 active patients developed short-lived fever.

- There was no evidence of myocarditis based on clinical, ECG or enzymatic evaluation at Week 1 to Week 12.
- Liver enzymes (SGOT and SGPT) increased modestly and transiently in a few patients in each cohort. The rises in all but two patients were less than twice the upper limit of normal. In one patient (patient #78002) the rise was considerable, occurring at Week 1 and returning to normal at Week 4 (see table below). The patient remained asymptomatic and her bilirubin levels were normal.
- In the other patient (patient #77026) one SGPT value only (93 IU/mL) was greater than twice the upper limit of normal.
- No significant trends were seen for other non-serious adverse effects or laboratory parameters.
- Serious adverse events occurred in 11 patients (10 active and 1 placebo [patient #75007]). These are tabulated below.
- Of significance, there were six patients (5 active and 1 placebo) with unstable angina, one of whom died (Patient # 80001, while awaiting CABG surgery, had a cardiac arrest and could not be resuscitated), one patient with carotid artery stenosis and one patient with colon and kidney cancer. Although the investigators reported that the coronary events were unlikely to be related to the gene product it is the sponsor's view that only a huge database with placebo control can allow a realistic interpretation regarding relationship between vascular events and gene product on the basis of an increase in plaque size. Also, with a 1: 3 ratio of placebo to active we would expect a higher number of side effects in the active group.
- Patient #77032 experienced blood in the stools. A diagnosis of cancer of the colon and kidney with secondaries in the liver, lung and adjacent nodes was made. Although the gene product could not have been the cause of the cancers, it is difficult to be certain whether the gene product did not play a role in altering the clinical presentation. A large-sized database and a lengthy follow-up is necessary to address this question.
- Antibody titers (IGG and neutralizing were increased at baseline in some patients and increased in some patients following gene product administration. The increase following gene product administration were dose dependent. On preliminary review, anti-ischemic effects and adverse events did not appear to be related to raised values at baseline or to increased values following gene product administration.
- Adenoviral infectious unit assay showed the presence of the adenoviral vector in the pulmonary artery blood (drawn during gene product administration) and in a few patients in the venous blood (drawn one hour post gene product administration). The amount of virus present in the pulmonary artery was dose dependent increasing with higher doses. In the 5th dose group (3.2×10^{10} VP), almost all patients had positive assays.
  Because of increased viral presence in the pulmonary artery at the 5th dose group (3.2×10^{10} VP) and because of the incidence of fever 4/9 patients (in 3 fever occurred within 24 hours of gene product injections) it was decided not to evaluate the 6th dose group (10^{11} VP). (We were cautious regarding the possibility of an elbow effect seen

in some of the adenoviral gene trials [using higher doses] presented at the December 1999 RAC meeting).

- Serum FGF-4 evaluations where available did not show detectable levels at any time point (limit of detection 50 pg/mL).

The purpose of the extension was to gain experience regarding feasibility and safety of administering Ad5FGF-4 in patients with patent bypass grafts so that this population could be included in subsequent studies. Twelve patients have been enrolled thus far (9 received Ad5FGF-4 10^{10} VP and 3 received matching placebo) and followed up for varying periods. Further enrollment in the study has been stopped.

The product administration into grafts was undertaken without problems and there were no immediate safety concerns. Anti-ischemic effect evaluation will be formally undertaken when all 12 patients have completed their 12 week follow-up. The fourth dose group of Ad5FGF-4 [10^{10} VP] was chosen for further evaluation in the perfusion study.

The primary objective of the perfusion study entitled "A Randomized, Double-Blind, Placebo Controlled Study to Evaluate the Effect of Ad5FGF-4 on Myocardial Perfusion Defect Size and Safety in Patients with Stable Angina" (OBA # 0006-403 is to determine whether Ad5FGF-4 (1.0 X 10^{10} viral particles) can significantly decrease the adenosine-induced ischemic left ventricular perfusion defect size as compared to placebo using quantitative ^{99m}Tc-sestamibi SPECT. The efficacy of Ad5FGF-4 is thought to be based on its ability to stimulate angiogenesis and thus increase blood flow to ischemic myocardium A reduction in the reversible (ischemic) perfusion defect size would imply an improvement in myocardial perfusion brought about by angiogenesis resulting from Ad5FGF-4.

The quantity of ischemic myocardium has been shown to inversely correlate with clinical outcome, that is, the greater the quantity the worse the outcome. The size of a reversible myocardial perfusion defect following a ^{99m}Tc-sestamibi SPECT study has been found to correlate well with subsequent clinical outcome, that is, the smaller the defect the better the outcome. In this model the size of the reversible perfusion defect is a surrogate for the quantity of ischemic myocardium

The ability of Ad5FGF-4 to decrease the size of a reversible ^{99m}Tc-sestamibi perfusion defect following adenosine infusion will be taken as a surrogate of the drug's ability to decrease the amount of ischemic myocardium, thus presumably influencing clinical outcome in a favorable manner.

Additional objectives arc as follows:

1. To evaluate the effect of Ad5FGF-4 on change in angina severity as measured by the investigator's assessment of the patient's Canadian Cardiovascular Society Class (CCS)

2. To evaluate the effect of Ad5FGF-4 on the change in frequency of angina1 attacks.

3. To evaluate the effect of Ad5FGF-4 on the change in weekly consumption of sublingual PRN NTG taken to relieve angina attacks.

4. To evaluate the effect of Ad5FGF-4 on the change from Baseline to Week 8 of the left ventricular stress perfusion count severity as measured by SPECT imaging.

5. To evaluate the effect of Ad5FGF-4 on the change in weighted score for the Duke Activity Status Index from Baseline to Week 8.